

Response of the Melon Fly Parasitoid *Pysttalia fletcheri* (Hymenoptera: Braconidae) to Host-Habitat Stimuli

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Cohorts of mass-reared adult female Pysttalia fletcheri, parasitoids of the melon fly (Bactrocera cucurbitae), were exposed to host-plant stimuli in a laminar airflow wind tunnel to analyze the cues used in host-habitat finding. Parasitoids hovered twice as frequently around plastic zucchini models emitting fresh cucumber odor as around models emitting clean air. The odor of decaying pumpkin was even more attractive, resulting in over a 10-fold increase in hovering, a 50-fold increase in landing, and a 150-fold increase in host-searching and probing behaviors compared to clean air. Fresh cucumber leaf odors were not attractive to the parasitoids, but decomposing leaves elicited a strong increase in hovering, landing, and searching behaviors. Plastic leaves which visually simulated cucurbit foliage did not in themselves significantly alter orientation behaviors, but the combination of leaf visual stimuli plus decaying leaf odors caused strong increases in hovering, landing, and searching. Fresh pumpkin odor and the odor of yeast-inoculated pumpkin were not as attractive to parasitoids as decaying leaf odors. Yeast isolated from decaying pumpkin and cultured on various sterile media were not substantially more attractive than clean air.

KEY WORDS: fruit fly; melon fly parasitoid; *P. fletcheri*; host-habitat finding; wind tunnel.

INTRODUCTION

The opiine braconid parasitoid *Pystallia fletcheri* (Silvestri) was introduced to Hawaii from India in 1916 for biological control of the the melon fly *Bactrocera*

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cucurbitae (Coquillett) (Nishida, 1956). The parasitoid became well established and spread throughout the Hawaiian Islands and is an important mortality factor for melon fly, particularly in wild (noncultivated) hosts such as bittermelon (*Mimordica charantii* L.). However, commercial crops of cucurbits (i.e., cucumber, *Cucumis sativa* L.; and zucchini, *Cucurbita pepo* L.) and tomatoes (*Lycopersicon esculentum* Mill.) remain heavily infested by the melon fly (Nishida and Bess, 1957). Consequently, researchers have attempted to develop efficient mass-rearing and release techniques in order to conduct augmentative releases of *P. fletcheri* for enhanced biological control of melon fly in commercial crops (Wong and Ramadan, 1992; Purcell *et al.*, 1994; Messing *et al.*, 1995).

Successful augmentative biological control with any natural enemy depends on a thorough knowledge of the behavioral ecology of the released species, particularly with regard to dispersal and host finding. However, there is relatively little information available regarding the behavior of *P. fletcheri* as it relates to host-habitat finding, host finding, and host acceptance. Nishida (1956) studied oviposition behavior of *P. fletcheri* in small glass jars and demonstrated the basic attractiveness of some host plant tissues to the parasitoids. Other than this work and bionomic research specifically related to mass-rearing (e.g., Wong and Ramadan, 1992), there have been no other studies of *P. fletcheri* behavior. We therefore conducted a series of experiments in a laminar airflow wind tunnel to analyze the response of adult, mated *P. fletcheri* females to combinations of visual and olfactory stimuli simulating those found in the field.

METHODS

Insects

P. fletcheri was reared at the USDA-ARS Tropical Fruit and Vegetable Research Laboratory in Honolulu, Hawaii, according to the methods of Wong and Ramadan (1992). All wasps were reared on melon flies using artificial (wheat-based) diet, containing no fruit or leaf materials. Parasitized puparia were shipped from the rearing facility to a laboratory in Hilo, Hawaii, on a weekly basis, where they were transferred to 30 × 30 × 30-cm wood and screen cages for emergence. Emerging adults were given water and honey ad libitum and held in a room at 20 ± 1°C. and 68 ± 5% RH, with indirect natural light. Neither fruit or leaf odors nor any green or yellow colors were present in the rearing room.

Wind Tunnel

All experiments were conducted in a laminar airflow wind tunnel in a separate laboratory, as described by Jang and Light (1991). The tunnel was

constructed of tempered glass and measured 0.9 m tall \times 0.9 m wide \times 2.8 m long, with intake and exhaust fans housed at the ends in sheet metal ducts. Temperature during the experiments ranged from 25.5 to 28.0°C. (mean = $26.7 \pm 0.1^\circ\text{C}$). Relative humidity ranged from 50 to 61% (mean = $54.4 \pm 3.4\%$). Wind inside the tunnel was pulsed on and off at 1-min intervals by an electronic timer to simulate gusty conditions occurring in the field, because parasitoid responsiveness is greater in intermittent than in constant wind (R.H.M., unpublished). Air speed in the tunnel (as measured with a Model 1650 Anemometer, TSI Inc., St. Paul, MN) ranged from 0.13 to 0.20 m/s.

Light inside the tunnel was generated by 6 Chroma-50 fluorescent bulbs (chromaticity, 5000 K; length, 2.5 m; manufactured by General Electric Inc., Cleveland, OH) which spanned the length and width of the top of the tunnel. Light intensity was 3940 lux at the center of the tunnel floor. A sheet of white paper was placed on the bottom of the tunnel; the sides were left uncovered. Initial flights of the parasitoids from the release platform appeared inhibited by the light, until we placed irregular cardboard cutouts on the top of the tunnel to provide shadows beneath. These cutouts modified light levels such that they varied from 1740 to 2780 lux across the tunnel floor. This improved flight activity of the wasps with or without odor source.

Experiment 1a: Response to Odor of Fresh Cucumber

Twenty-five adult female parasitoids per trial were aspirated from the holding cages and transferred as a group to the wind tunnel in a single 5-dram plastic vial. The vial was sealed at one end with tissue paper and at the other end with a plastic cap. Parasitoids were released by placing the vial parallel to the direction of wind flow on a platform at the downwind end of the tunnel, then gently removing both the cap and the tissue without shaking or otherwise disturbing the parasitoids. This allowed air to flow through the vial, so that egress was a response to light and/or odor stimuli, not to mechanical disturbance. The release platform was 42 cm above the chamber floor and 2.0 m downwind from the odor sources near the tunnel's intake hood.

Each trial was a two-choice assay in which the parasitoids could fly to one of two focused air streams of equal strength emanating from 0.4-mm-diameter holes in green plastic models of zucchini (ca. 18 cm long \times 4 cm in diameter) suspended by thin wires about 45 cm from the top of the flight tunnel. One model (the control) emitted clean, odorless, filtered, compressed air. The other model (treatment) emitted odors from one or two washed, supermarket-purchased cucumbers (203–489 g/trial) which were manually punctured 200 times each over the entire surface of the fruit with a 1-mm-diameter steel probe. For each trial, fruit were placed in one of two identical glass chambers adjacent to the tunnel. Compressed air was pumped through Teflon tubing at 75 ± 2 cm/min through the glass chambers (one empty, one with fruit) and then through

the respective plastic zucchini models in the upwind end of the tunnel. Cucumbers were not infested by *B. cucurbitae* or other insects. Each trial lasted 31 m.

Parasitoids tested in this experiment ranged in age from 8 to 14 days posteclosion, were always naive (e.g., no prior exposure to fruit odors or larvae), and were discarded after each assay. The position of control and treatment models was alternated horizontally in successive trials to control for positional bias. All trials were conducted between 0900 and 1600 h over a 6-day period. The experiment was replicated 14 times.

Experiment 1b: Response to Odor of Decomposing Pumpkin

This test used methods identical to those in Experiment 1a, with one exception: the treatment fruit model emitted the odor of decomposing pumpkin fruit (*Cucurbita maxima* × *C. mochoata* L., hybrid “kabocha”; 207–349 g/trial). Pumpkins were maintained under ambient outdoor conditions in an opaque plastic garbage can, with weekly additions of fresh slices and removal of completely decomposed slices. The experiment was replicated 23 times over a 7-day period.

Experiment 2a: Response to Fresh and Decomposing Leaf Odors

This was a two-choice assay identical to Experiment 1, except that the treatment model emitted odors from either freshly picked cucumber leaves (73–78 g/trial; four replicates) or decomposing cucumber leaves (63 g/trial; four replicates) that had been frozen for at least 24 h, removed from the freezer on the morning of the assay, and kept at room temperature during the duration of all trials. Parasitoids for this and all subsequent experiments were 11–13 days old.

Experiment 2b: Response to Leaf Odors Plus Leaf Visual Stimuli

This test used methods identical to those in Experiment 2a, except that a branch of artificial plastic leaves (ca. 30 cm tall × 40 cm wide) resembling cucurbit foliage was placed within 5 cm of the treatment zucchini model emitting decomposing leaf odors to provide visual cues simulating plant foliage. This experiment was replicated 10 times.

Experiment 2c: Response to Combined Leaf and Fruit Odors

This two choice assay used methods identical to those in Experiment 2b with one change: the compressed air was first passed through a primary chamber which contained decaying pumpkin fruit. The effluent line from this chamber was then split into two separate lines, each of which passed through a separate secondary chamber. This ensured that equal quantities of identical decaying

pumpkin odor passed through each fruit model. One of the secondary chambers was used to introduce leaf odors into the air stream; the other remained empty. Thus, decaying pumpkin odor was used as the control, and the treatment was decaying pumpkin odor combined with decaying leaf odor. This experiment was replicated 10 times.

Experiment 3a: Response to Odors of Fresh and Yeast-Inoculated Pumpkin

This test used methods identical to those in Experiment 1, except that the control fruit model emitted decomposing cucumber leaf odors, while the treatment model emitted odor of either freshly sliced pumpkin (15 replicates) or pumpkin slices deliberately inoculated with yeast (15 replicates). The yeast inoculum was isolated from a single colony which predominated on pumpkin slices kept outdoors as described in Experiment 1. (The yeast was identified as *Candida krusei*, the non-ascospore-forming state of *Issatchenkia orinetalis* Kudryavtsev, by the Centraalbureau voor Schimmel-cultures, The Netherlands.) *C. krusei* yeast cells were cultured in petri plates on PDA medium (Difco, Detroit, MI) at 24°C in continuous fluorescent light and placed on pumpkin slices 60–120 h before each assay. Inoculation was not done in a sterile environment, so other airborne organisms also appeared on the pumpkin, but the yeast culture predominated in all cases.

Experiment 3b: Response to Odors of Cultured Yeast

This test used methods identical to those in Experiment 1, except that for the first eight replicates, the treatment model emitted odors from two to four 100-cm-diameter petri plates of *C. krusei* yeast culture grown on potato extract, dextrose, and agar medium (PDA), while the control model emitted clean air. The next four replicates tested odors from the same yeast grown on potato extract and dextrose broth (PDB) versus clean air. The last four replicates tested odors from the same yeast cultured on peptone, yeast extract, malt extract, and dextrose agar (PYMDA) versus yeast cultured on PDA.

Behavioral Recording Methods

For all experiments, behavior of parasitoids was recorded using Observer software (v. 3.0, Noldus Information Technology, Wageningen, The Netherlands) on a computer acting as a multichannel event recorder. The experimenter stood about 1 m to the side of the wind tunnel, and both sides of both fruit models were made visible by the use of a planar mirror on the interior of the left side of the tunnel. Four distinct behaviors were recorded. (A) *Hovering* was stationary flight within 10 cm of the zucchini or vegetation model. Linear flights

past the models with no indication of orientation to the fruit or foliage were not included. In some instances a parasitoid appeared to detect the odor plume from >0.5 m downwind and to make a very slow and controlled flight directly to the odor source. Since these oriented flights were distinct and easily recognized, they were recorded as hovering when wasps entered the zone <0.1 m from the model. Data were recorded as the total number of wasp-seconds (for all females in the tunnel) spent hovering per trial. (B) *Landing* was alighting on a fruit model, regardless of the duration of the preceding flight. Landings on the foliage models, which occurred frequently, were not recorded. (C) *Searching* was walking on the fruit model with the antennae vibrating and contacting the surface. This activity was recorded for each discrete walk of two or more steps. (D) *Probing* was insertion of the ovipositor into one of the air holes in the fruit model. We discuss *attraction* as any increase in the frequency of the above-mentioned behaviors, which could be a combination of taxes (orientation) and kineses (arrestment), that would likely lead to an increase in successful host-finding and subsequent parasitism.

Data Analyses

Data from the Observer file were translated into a SAS data set in a static record format, in which each observation became a data record with all associated treatment and environmental variables attached. The data were merged so that horizontal position (right or left in the wind tunnel) and treatment were both variables. These data were then subjected to frequency analysis by the SAS procedure FREQ, to determine the frequency of each behavior by date, time of day, and treatment. Frequencies were then analyzed using the GLM procedure. Both parametric analysis of variance and nonparametric Friedman's analysis of the data ranks were applied. Tukey's studentized rank test was used to compare treatment means where Friedman's analysis is reported.

The analyses of experiments 1 and 4 use data from simple two-choice assays, while the analyses for experiments 2 and 3 merge data from similar, related assays into a single ANOVA over the different treatments. We justify this merger on the basis of the uniformity of the experimental conditions from assay to assay and the genetic and epigenetic relatedness of a parasitoid cohort during each experiment. We make no attempt to conduct statistical comparisons between discrete experiments.

RESULTS

Experiment 1a

Parasitoids given a choice between a zucchini model emitting filtered air and one emitting air mixed with the odor of fresh cucumber showed significantly

Table I. Effect of Fresh Cucumber Odor on Orientation of *P. fletcheri* Females

	Reps	Hovering ^a	Landing	Searching	Probing
Control: clean air	14	7.64 a	0.0	0.0	0.0
Treatment: fresh cucumber	14	15.71 b	0.0	0.0	0.0
GLM analysis of variance ^b					
<i>F</i>		4.71	NA	NA	NA
<i>P</i>		0.0401			

^aEach number indicates the total number of wasp-seconds (for all females) spent hovering per trial. Numbers followed by the same letter within each column are not significantly different using Tukey's pairwise comparison of data ranks (SAS, 1988; $P \geq 0.05$).

^b*F* tests (type III) are based on two-way analysis of variance (GLM), with treatment and position as the first and second levels in the analysis, respectively. Significant *P* values are boldfaced.

higher rates of hovering around the latter (Table I). However, no landings (and hence neither searching nor ovipositor probing) occurred during any of the 14 trials using fresh cucumber as the odor source.

Experiment 1b

With decaying pumpkin as the odor source, parasitoids showed significantly higher levels of hovering, landing, and searching activity on the treatment models than on those emitting clean air (Table II). Under identical experimental conditions as in Experiment 1a, hovering activity was about nine fold higher around models emitting decaying pumpkin odor as around models emitting fresh cucumber odor.

Experiment 2a

The odor of fresh cucumber leaves elicited no response from female *P. fletcheri* in the flight tunnel (Table III). However, there was a strong response

Table II. Effect of Decaying Pumpkin Odor on Orientation of *P. fletcheri* Females

	Reps	Hovering ^a	Landing	Searching	Probing
Control: clean air	23	10.30 a	0.22 a	0.26 a	0.00 a
Treatment: rotting pumpkin	23	138.52 b	12.78 b	41.39 b	0.35 a
GLM analysis of variance ^b					
<i>F</i>		27.60	20.39	18.49	1.55
<i>P</i>		0.0001	0.0001	0.0001	0.2207

^aEach number indicates the total number of wasp-seconds (for all females) spent hovering per trial. Numbers followed by the same letter within each column are not significantly different using Tukey's pairwise comparison of the data ranks (SAS, 1988; $P \geq 0.05$).

^b*F* tests (type III) are based on two-way analysis of variance (GLM), with treatment and position as the first and second levels in the analysis, respectively. Significant *P* values are boldfaced.

Table III. Effect of Leaf Odors on Orientation of *P. fletcheri* Females

	Reps	Hovering ^a	Landing	Searching	Probing
Control: clean air	8	4.88 a	0.13 a	0.00 a	00.00 a
Treatment					
Fresh leaf odor	4	0.00 a	0.00 a	0.00 a	00.00 a
Decomposing leaf odor	4	396.50 b	35.00 b	104.50 b	00.00 a
GLM analysis of variance ^b					
<i>F</i>		47.94	20.21	14.00	NA
<i>P</i>		0.0001	0.0003	0.0013	NA

^aEach number indicates the total number of wasp-seconds (for all females) spent hovering per trial. Numbers followed by the same letter within each column are not significantly different using Tukey's pairwise comparison of the data ranks (SAS, 1988; $P \geq 0.05$).

^b*F* tests (type III) are based on two-way analysis of variance (GLM), with treatment and position as the first and second levels in the analysis, respectively. Significant *P* values are boldfaced.

to the odor of decomposing leaf tissue, including highly significant increases in hovering, landing, and searching behavior.

Experiment 2b

The addition of leaf visual stimuli to the model emitting pumpkin odors did not result in any increase in landing, searching, or probing behaviors for *P. fletcheri* in the flight tunnel. There was, however, a three-fold increase in the amount of hovering around the model with artificial leaves, although large amounts of variation between trials resulted in a lack of statistical significance for this difference (Table IV).

Table IV. Effect of Leaf Odor and Visual Model on Orientation of *P. fletcheri* Females

	Reps	Hovering ^a	Landing	Searching	Probing
Control: rotting pumpkin odor	20	222.1 a	17.35 a	29.05 a	00.20 a
Treatment					
Pumpkin odor + leaf visual	10	641.0 a	14.10 a	34.60 a	00.10 a
Pumpkin & leaf odor + leaf visual	10	1806.6 b	37.50 b	84.90 b	00.00 a
GLM analysis of variance ^b					
<i>F</i>		39.62	4.99	8.23	2.53
<i>P</i>		0.0001	0.0126	0.0012	0.0946

^aEach number indicates the total number of wasp-seconds (for all females) spent hovering per trial. Numbers followed by the same letter within each column are not significantly different using Tukey's pairwise comparison of the data ranks (SAS, 1988; $P \geq 0.05$).

^b*F* tests (type III) are based on two-way analysis of variance (GLM), with treatment and position as the first and second levels in the analysis, respectively. Significant *P* values are boldfaced.

Experiment 2c

Combining decaying pumpkin odor with leaf odors and leaf visual stimuli resulted in a substantial increase in hovering, landing, and searching behaviors of the parasitoids (Table IV). Hovering behavior around zucchini fruit plus leaf models emitting pumpkin odor increased almost three-fold when the odors of decomposing leaves were added, while landing and searching behaviors both increased more than two-fold.

Experiment 3a

Parasitoid responses to both fresh pumpkin odor and odor from pumpkins which had been inoculated with *C. krusei* 3–6 days before each assay were lower than the response to decomposing leaf tissues (Table V). The comparatively weak response of *P. fletcheri* to the odor of pumpkin that had been inoculated with yeast was unexpected. The yeast cultures had been allowed up to 6 days to colonize the pumpkin substrate but were unable to produce the quantity or quality of attractive odors previously associated with decaying pumpkin.

Experiment 3b

C. krusei was the predominant microorganism from the culture of decaying pumpkin described in Experiment 2. We tested the attractiveness of this yeast cultured on noncucurbit substrates to determine if the organism itself was attractive, or if its growth on cucurbit substrate was necessary to attract the parasitoids.

Table V. Effect of Fresh and Yeast-Inoculated Pumpkin Odors on Orientation of *P. fletcheri* Females

	Reps	Hovering ^a	Landing	Searching	Probing
Control: decomposing leaf odor	30	56.1 a	5.03 a	12.53 a	0.0
Treatment					
Fresh pumpkin	15	27.0 b	2.87 b	6.87 b	0.0
Yeast inoculated pumpkin	15	20.5 b	2.80 b	5.40 b	0.0
Friedman's analysis ^b					
<i>F</i>		9.13	11.31	11.28	NA
<i>P</i>		0.0004	0.0001	0.0001	NA

^aEach number indicates the total number of wasp-seconds (for all females) spent hovering per trial. Numbers followed by the same letter within each column are not significantly different using Tukey's pairwise comparison of the data ranks (SAS, 1988; $P \geq 0.05$).

^b*F* tests (type III) are based on Friedman's two-way analysis of block designs, with treatment and position as the first and second levels in the analysis, respectively. Significant *P* values are bold-faced.

Table VI. Effect of Cultured Yeast Odors on Orientation of *P. fletcheri* Females

	Reps	Hovering ^a	Landing	Searching	Probing
Control: clean air	12	2.33 a	0.08 a	0.00	0.00
Treatment					
PYMDA yeast culture	4	1.00 a	0.00 b	0.00	0.00
PD broth yeast culture	4	1.50 a	0.00 b	0.00	0.00
PDA yeast culture	12	4.41 a	0.17 a	0.42	0.00
Friedman's analysis ^b					
<i>F</i>		1.52	3.66	5.49	NA
<i>P</i>		0.2068	0.0080	0.0007	NA

^aEach number indicates the total number of wasp-seconds (for all females) spent hovering per trial. Numbers followed by the same letter within each column are not significantly different using Tukey's pairwise comparison of the data ranks (SAS, 1988; $P \geq 0.05$).

^b*F* tests (type III) are based on Friedman's two-way analysis of block designs, with treatment and position as the first and second levels in the analysis, respectively. Significant *P* values are bold-faced.

When cultured on petri plates containing PDA, *C. krusei* was marginally attractive to *P. fletcheri* females, while broth cultures and PYMDA cultures of the yeast were not at all attractive (Table VI).

DISCUSSION

P. fletcheri normally stings its host, the melon fly, when the fly is in the third larval instar, just prior to its emergence from the fruit to pupate. By the time these larvae are ready to emerge, host-plant tissues are usually in a state of moderate to advanced decay as the result of larval feeding and defecation. Our data show that the parasitoids use chemical stimuli associated with this plant decay as cues for host-habitat finding.

Fresh cucumber odors had only a slight effect on parasitoid behavior (Table I), indicating that fresh cucurbit volatiles may be partially attractive (resulting in increased arrestment, or hovering) but do not appear sufficient to cause the parasitoids to land (a necessary prerequisite to host-finding and oviposition). When a parasitoid moves upwind in the flight tunnel and approaches an odor-emitting fruit model, this hovering behavior is usually quite distinct, and appears to be an intermediate step allowing further integration of information before the wasp makes the decision to land. In a two-step process, volatiles from fresh (undamaged) plants may help parasitoids find a suitable patch of potential host habitat, while volatiles from decaying tissues may indicate with a higher likelihood an actual host habitat (i.e., infestation by melon flies).

The melon fly is unique among the pest fruit flies established in Hawaii in that it sometimes oviposits into, and emerges from, plant stems rather than fruit

tissues (Nishida and Bess, 1957). We hypothesize that the strong response of the parasitoids in our experiments to decomposing leaf odors (Table III) is a reaction to chemical stimuli that the leaves have in common with stem tissues. Nishida (1956) demonstrated that stem tissues of cucumber, watermelon, pumpkin, and tomato were more attractive than the fruits of these plants to adult female *P. fletcheri*, even though, for example, melon fly does not develop in tomato stems in the field. Thus, it is likely that nonspecific volatiles form a basis of this attraction. These may be comparable to the green leaf volatiles (GLVs) shown to attract other braconid species to plants damaged by leaf-feeding Lepidoptera (Whitman and Eller, 1990). Odors from rotting leaf or stem tissues are fundamentally different from odors of rotting fruit, at least in part because of the difference in microorganisms involved: leaf rots are caused primarily by bacteria (e.g., *Klebsiella*, *Enterobacter*), while fruits are more frequently attacked by yeasts and other fungi (e.g., *Monolina*, *Penicillium*) responding to their higher concentrations of sugars and starches. During our trials, only decaying fruit odors elicited ovipositor probing behavior on the part of the parasitoids.

Although *P. fletcheri* relies heavily on olfactory cues to find the microhabitat of its hosts, it appears that there is also a visual component to habitat-finding that is integrated into the overall behavioral process, at least in the initial (hovering) stage of host-searching. Leaf visual models resulted in substantial increases in hovering behavior when paired with fruit odors; however, because of the high assay-to-assay variation in behavior in the wind tunnel, this increase was not statistically significant (Table IV). The additive effect of leaf visual cues, leaf odors, and fruit odors becomes more apparent in the large increase in hovering, landing, and searching behaviors on plastic fruit models (Table IV). Studies with the related opiine braconid parasitoid *D. longicaudata* have also shown that visual cues can be used by females in the location of host fruits, although this faculty is more pronounced in the absence of olfactory stimuli (Messing and Jang, 1992).

The response to the odor of cucurbits in *P. fletcheri* is apparently innate. All individuals used in our experiments were reared on fly hosts in a wheat-based artificial diet, yet they responded strongly to cucurbit odors although they had no prior experience with these volatiles.

The interaction of naturally occurring decay organisms with fly-infested cucurbit fruits in the field may result in a wide array of odors, although certain common volatiles will probably be emitted by all decaying fruits. Greany *et al.* (1977) reported that both acetaldehyde and ethanol, common by-products of fruit fermentation, were attractive to female *D. longicaudata*. In our wind tunnel experiments, we observed no response of *P. fletcheri* to 5, 10, or 20 ml of acetaldehyde in three separate assays, although it is possible that the rates we tested were too high and lower rates might prove attractive. Also, pumpkins

inoculated for 3–6 days with common colonizing yeasts (*C. kraussi*) were not as attractive to the parasitoids as decomposing leaf tissues. Our experimental design was inadequate to determine whether this was strictly a function of insufficient time for the yeasts to break down the plant tissues or was due to a lack of other microorganisms contributing to the decay process. *C. kraussi* cultured on three sterile media did not produce volatiles substantially attractive to the parasitoids, hence it is likely that cucurbit plant tissues are a necessary substrate for production of attractive volatiles. Further studies of the interactions of microorganisms and plant tissues, particularly those responsible for rotting leaf odors, are needed to establish a basis for possible manipulation of the behavior of these parasitoids with semiochemicals in the field.

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